## REMARKS

Each rejection raised by the Examiner is addressed separately below. In view of the remarks discussed below, Applicants respectfully request reconsideration of the merits of this patent application.

A Declaration under 37 CFR §1.132 of Dr. Glauco Tocchini-Valentini, the named inventor in this application and an expert in the field of the present invention, is attached hereto. Consideration of this Declaration is respectfully requested.

Favorable reconsideration and allowance of this application is respectfully requested.

### Rejections Under 35 U.S.C. § 35 U.S.C. §103

Claims 1 and 4-17 are rejected under 35 U.S.C. 103(a) as being obvious over Abelson et al. (The Journal ofBiological Chemistry, 1998,273:12685-12688) in view of Diener et al. (Molecular Cell, 1998, 1:883-894) and Reyes et al. (Analytical Chemistry, 1987, 166:90-106).

The Examiner alleges that it would have been obvious to one of one or ordinary skill in the art to use a conserved, common mechanism for recognizing non-tRNA RNA substrates having a BHB motif to cleave a desired target nucleic acid in view of Abelson and Reyes. The Examiner alleges that Abelson teaches that tRNA endonuclease inherently cleaves the BHB structure of a tRNA substrate and that Reyes teaches cleaving an artificially-synthesized, non-tRNA oligonucleotide substrate that contains the BHB motif, each containing the G/A and U/A dinucleotides. The Examiner argues that one of skill would be motivated to do so because Abelson teaches that the BHB-motif structure-based cleavage of a target RNA oligonucleotide substrate is a conserved, absolute biological mechanism of a tRNA splicing endonuclease which can cleave any "universal" substrate containing the BHB motif, and Reyes teaches that tRNA splicing endonuclease-mediated tRNA cleavage was demonstrated to occur in any synthetic oligonucleotide substrate having proper structures that can be recognized by the tRNA splicing endonuclease. Applicants disagree, and have submitted a Declaration under 1.132 by named inventor Dr. Glauco P. Tocchini-Valentini (hereafter Declaration) in support thereof.

The Abelson reference teaches that "it is likely that what has been conserved since the divergence of the Eukary and the Archaea is the endonuclease active site and the means to array two of them in a precise and conserved spatial orientation." (Abelson, p. 12688, last paragraph). Abelson cites as support for this "the results of Tocchini-Valentini and co-workers" (specifically,

Fabbri et al., Science 280 (1998) 284-286, which has been addressed in previous responses) "where it is demonstrated that both the eukaryl and archaeal endonucleases can accurately cleave a universal substrate containing the BHB motif." (Abelson, p. 12688, last full paragraph). Accordingly, when the Examiner cites Abelson as teaching that the BHB-motif structure-based splicing or cleavage of a target RNA oligonucleotide substrate is a conserved, absolute biological mechanism of a tRNA splicing endonuclease which can cleave the "universal" BHB substrate, she is actually citing Fabbri.

The Examiner notes that "the teachings of Abelson that a eukaryotic or archaeal tRNA splicing endonuclease is capable of targeting any target comprising a conserved structural motif are not purely based on the teachings of Fabbri et al." (Office Action, pg. 3). Applicants submit that Examiner has provided no evidence of this conclusion. The Abelson reference provides a "mini-review" of the state of the art of tRNA splicing in 1998 (title, abstract). Every statement in Abelson is a summary of previously printed material, and each statement is cited accordingly. Applicants submit that the conclusions made in the Abelson reference are based on the references cited therein. Citing the Fabbri reference as "further support" for this evolutionary pathway cannot be used the render the present invention obvious, given that, as discussed below, the Fabbri reference does not teach or suggest the elements of the present invention.

As discussed in previous responses, Applicants again acknowledge that Fabbri disclosed that pre-tRNAs (i.e., a tRNA structure having (1) a 5'-terminal phosphate group; (2) an acceptor stem comprising a seven base pair stem made by the base pairing of the 5'-terminal nucleotides with the 3'-terminal nucleotides; (3) a CCA tail at the 3' end; (4) a D loop comprising a four base pair stem ending in a loop; (5) an anticodon loop comprising a five base pair stem whose loop contains the anticodon; and (6) a T loop comprising a five base pair stem) and mini-substrates having whole, cis-formed BHB motif (including a terminal loop) can be cleaved by not only archaeal endonucleases, but also by eukaryal tRNA endonucleases (see FIGS. 1-2 of Fabbri). However, Fabbri does not contemplate or disclose to one of ordinary skill in the art that a trans-formed BHB motif (lacking a terminal loop) could be cleaved by eukaryal tRNA endonucleases (see, e.g., FIGS. 4 and 13 of the application for transformed BHBs). Thus, in contrast to Fabbri, the claimed methods do not require all the structures present in pre-tRNA for cleavage, which allows one to advantageously cleave non-tRNA molecules (Declaration, para. 3-5).

As previously explained, Fabbri is strictly directed toward cleavage of pre-tRNAs and mini-substrates having a whole, cis-formed BHB motif and does not contemplate or disclose trans-formed BHBs. Similary, Abelson is also strictly directed toward cleavage of pre-tRNAs and mini-substrates having a whole, cis-formed BHB motif and does not contemplate or disclose trans-formed BHBs. At the time of Fabbri and Abelson, it was neither known, nor predictable that a trans-formed BHB could result in cleavage of an RNA molecule by eukaryal tRNA endonucleases (Declaration, para. 6). In contrast, the claimed methods are directed toward Applicants' surprising finding that such enzymes can recognize and cleave trans-formed structures having only a BHB motif. Applicants submit that neither Fabbri, nor Abelson, teach or suggest Applicants' surprising finding that a trans-formed BHB could result in cleavage of an RNA molecule by eukaryal tRNA endonucleases and that such enzymes can recognize and cleave trans-formed structures having only a BHB motif (Declaration, para. 10).

Nothing in Diener or Reyes corrects this deficiency. Diener is directed to determining the solution structure of the BHB motif by NMR. The Examiner cites Diener as showing the motif-based splicing of a tRNA substrate because Fig. 1 of Diener shows that the 3' cleavage site of the BHB motif in pre-tRNA contains the U/A dinucleotide, wherein the cleavage occurs between the U and the A, and that the 5' cleavage site of the BHB motif in the pre-tRNA contains the G/A dinucleotide, wherein the cleavage occurs between the G and the A. However, like Fabbri and Abelson, Diener does not teach or suggest that a trans-formed BHB motif (lacking a terminal loop) could be cleaved by eukaryl tRNA endonucleases, as recited in the pending claims.

Reyes is cited by the Examiner as teaching a method of synthesizing an artificial RNA oligonucleotide substrate for in vitro splicing by a tRNA splicing endonuclease, where the artificial substrate has a BHB with the G/A and U/A dinucleotides (i.e., one bulge of the BHB has a guanine/adenine dinucleotide and the other bulge of the BHB has either a uracil/adenine dinucleotide or a thymine/adenine dinucleotide.) However, nothing in Reyes teaches that non-tRNA target RNA can be cleaved by eukaryl endonucleases when the BHB is present in this form.

Accordingly, as none of Abelson, Diener or Reyes, either alone or in combination, teach or suggest that non-tRNA target RNA can be cleaved by eukaryl endonucleases when the BHB has the G/A and U/A dinucleotides, this rejection should be withdrawn.

Summary

In view of the above remarks, reconsideration is respectfully requested. The application is believed to be in condition for allowance and allowance of the same is requested. If all the claims are not allowed, Applicant requests a telephone interview with the Examiner and his supervisor. Applicants have enclosed a Petition for Three Month Extension of Time and a Request for Continued Examination. The Commissioner is authorized to charge any fees under 37 CFR § 1.17 that may be due on this application to Deposit Account 17-0055. If further fees are necessary, please charge Deposit Account 17-0055. The Commissioner is also authorized to treat this amendment and any future reply in this matter requiring a petition for an extension of time as incorporating a petition for extension of time for the appropriate length of time as provided by 37 CFR § 136(a)(3).

Respectfully submitted,

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